10. NOTE ON THE MORPHOLOGY AND ENDOTROPHIC MYCORRHIZA OF RHIZANTHELLA GARDNERI, ROGERS, AND CERTAIN OTHER WESTERN AUSTRALIAN ORCHIDS.

(With Three Plates, X., XI., XII.)

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(1) **Rhizanthella Gardneri**, Rogers.

As has been shown by Rogers and Gardner in a recent number of the Proceedings of this Society,* the vegetative portions of this orchid are entirely subterranean. The plant is composed of a branching, roughly cylindrical, whitish rhizome, without roots. At the infrequent and faintly marked nodes occur short but wide-based white to dingy-coloured scales. The growing point produces a large bud which eventually expands into a bracteate capitulum of numerous small flowers. The lateral branches of the rhizome also each terminate in a flower-bud, but tend to be much narrower, more elongated and more thickly covered with bracts than the main or apical flowering stalk. The largest rhizome yet seen was about three inches long by one inch in diameter with lateral branches up to about four inches long by one quarter inch thick.

No portion of the plant has any chlorophyll development (or indeed the development of any pigment whatever with the exception of the capitulum bracts and the flowers themselves which may show a faint purplish colour especially on exposure to light). Supplies of organic matter must therefore be obtained in an already at least partially elaborated form. This is achieved through the agency of a mycorrhizal fungus on which the orchid must be considered to be parasitic.

Although devoid of roots the rhizome is fairly thickly covered towards the distal end with thick-based hairs which serve as a means of entry and of exit for the endotrophic mycorrhiza. Each of these hairs has a long, cylindrical, filamentous thin-walled apical cell and a large, wart-like multicellular base. (Fig. 6, Plate XII.) The rhizome has no well marked protective or strengthening tissues externally but simply a thin-walled, small-celled epidermis. Immediately within the epidermis lies the ground tissue in which occur a variable number of small vascular bundles. In certain material examined the vasculars were arranged at wide intervals in a circle (Fig. 2, Plate X.), but in other rhizomes the vasculars were scattered about through the ground tissue in the normal manner characteristic of monocotyledonous stems. In the somewhat limited amount of material available for examination, no starch could be found within the tissues but considerable numbers of fat or oil drops were present in the peripheral cells occupied by the fungus.

With the exception of the epidermis, the outermost cells to a depth of about ten cell layers contained an endotrophic mycorrhiza. (Fig. 3, Plate XI.) In most of the material the fungus occurred all around the periphery of the rhizome, but in some cases the invaded tissue did not constitute a completely

* ROGERS, R. S. A new Genus of Australian Orchid. Jour. Roy. Society of Western Australia, Vol. XV., pp. 1-5, 2 plates, 1928-29.

continuous band, being broken at intervals by small patches of uninfected cells. Infection appears to take place through the lumina of the filamentous apical cells of the thick-based hairs before mentioned. Not all of these apical cells contained the fungal filaments but in the majority a number of hyphae occurred. These ran more or less parallel to one another and branched infrequently. The hyphae in these cells had not become entangled, but had remained distinct from one another. In the cells at the base, however, they had become so much branched and so densely tangled together that the bases of the hairs appeared to the naked eye as minute black dots or sclerotia on the surface of the rhizome. (Fig. 6, Plate XII.) No case was seen where the fungus had entered or left the plant except through the apical cells of the thickbased hairs.

Both in the apical cells of the hairs and in the cells of the ground tissue the fungus was markedly septate (Fig. 4, Plate XI.), and could be seen branching very obviously at right angles. In unstained sections the fungal hyphae and the amorphous masses into which they eventually degenerate had a golden to brown colour, and the general appearance of the hyphae strongly suggested a species of *Rhizoctonia*.

In the innermost two or three rows or so of invaded cells in the material examined, the fungal hyphae had become closely clumped together and had passed into an amorphous golden-brown deeply staining mass to which the enlarged and deeply-staining nuclei of the host cells could often be seen closely applied. (Fig. 5, Plate XII.) The specimens examined had not flowered and in the majority of the infected cells the fungal hypae were winding very much about one another, branching frequently at right angles and closely enclosing the disorganised host nuclei. The fungus was wholly intracellular and consequently no arbuscles or sporangioles were present. No vesicles or spore like bodies were observed within the host cells. Where the hyphae came into contact with the host walls in passing through the tissues they had commonly formed swollen appressorium-like structures from each of which a very narrow peg-like process had grown out to penetrate the host wall. On the other side of the wall the hyphae had regained their normal dimensions. (Fig. 5. Plate XII.)

Attempts were made on several occasions to isolate the mycorrhizal fungus and on each occasion a particular species of *Aspergillus* was obtained. The growth characters on culture media with the exception of the colour of the mycelium closely resembled the type of mycelium found in the orchid. Mature seeds or uninfected rhizomes of the higher plant were not available, however, for infection experiments, and the relation or otherwise of the isolated fungus to the mycorrhizal condition is not known. A very suitable staining combination for the study of the mycorrhiza of *Rhizanthella* is Night Blue or Cyanin followed by Acid Fuchsin after fixing in Flemming's weaker solution.

(2)—Some Other Terrestrial Western Australian Orchids.

As a matter of interest and for purposes of comparison certain other Western Australian orchids were examined. These included *Caladenia flava* R.Br., *C. Patersoni* R.Br. var. *longicauda* (Lindl.), Rogers, *C. gemmata* Lindl., *C. sericea* Lindl., *Pterostylis turfosa* Endl., *Prasophyllum fimbria* Reichb., *Diuris longifolia* R.Br., *Lyperanthus nigricans*, R.Br., *Thelymitra crinita* Lindl. and *T. longifolia* Forst. All specimens were examined when in flower.

(a) **Caladenia** species.

As stated in Bentham's "*Flora Australiensis*" the Caladenias are terrestrial herbs with small tunicated underground tubers. Below the ground level the downward prolongation of the flowering axis can often be traced vertically to a depth of about three or four inches, at about which point one or more laterally-borne tubers are commonly found. These tubers on sectioning are found to show the scattered vascular arrangement and general structure typical of monocotyledonous stems. Beyond the point of attachment of these tubers the axis appears to lie more or less horizontally and often increases in diameter as it is traced backwards to a point where a further structure occurs which is apparently the original or parent tuber. In certain material of *C. Patersoni* var. *longicauda*, small roots arose from the point of attachment of the axis to the parent tuber. Apart from these small roots which may arise at the junction of the parent tuber and the axis of the new plant, no other roots seem to be formed.

It would be expected from the method of derivation of the main axis (arising as it does from a true stem-tuber) that the underground portion would have the structure of a stem. On sectioning, however, the anatomy is commonly found to be more like that of a root or at the least a much-modified stem. There is a thin-walled epidermis within which is a fairly extensive cortex region which is very obviously delimited from the vascular cylinder by an endodermis of one or more rows of cells (Fig. 1, Plate X.). The endodermal cells are made very obvious in either transverse or longitudinal section on account of the presence of large, branched, spiral thickenings. In the material of *C. Patersoni* var *longicauda* there was a four or more rowed endodermis ; *C. gemmata* had two to three rows ; *C. flava* had two rows, while *C. sericea* had one. (*C. flava* differed very markedly from the other species mentioned by having very large semicircular thickenings on the radial walls of the epidermal cells). In the material of *C. flava* the vascular cylinder had very much the structure of a woody dicotyledonous stem. There were some five or six separate vascular bundles arranged more or less in circular fashion around a well-marked "pith." The xylem elements were large and thick-walled while the phloem tissues were small celled and thin walled, and arranged in much the same manner as in a woody dicotyledonous root or stem. In *C. sericea* the vasculars were much more scattered and had more the arrangement of a typical monocotyledonous stem than in *C. flava*.

The exterior of the underground stem in all the Caladenias examined showed numerous multicellular wart-like hairs very much like those met with in *Rhizanthella*. They had this difference, however, that instead of merely a single elongated apical cell to each protuberance (as in *Rhizanthella*) the majority had a *number* of long filamentous cells produced by the outward prolongation of as many terminal cells. (Fig. 1, Plate X.) These provided the sole means of entrance and of exit for the mycorrhizal fungus which occurred in the cells of the cortex. In all the material examined the fungus was in the amorphous condition in the whole of the cells occupied with the exception of those of the hairs. (Fig. 1, Plate X.). No fungus was found in the tubers.

A peculiar feature of the underground portions of the Caladenias examined is the possession of a continuous brown sheath formed from organic matter, interwoven apical cells of the wart-like hairs, fungal hyphae, sharp-pointed spiral rods and certain peculiar net-like thickenings which appear to originate in the swollen portion of the stem immediately in advance of the old parent tuber. The sheath is very absorbent of moisture and may serve

the purpose of keeping the underground parts of the plant moist during periods of comparative soil dryness. Although it is closely applied to the underground stem it is quite distinct from it, except in the lower regions which lie between the old and the new tubers. The sheath can be readily removed from the stem above the new tubers without causing any damage to the plant tissues except for the breaking of many of the filamentous cells of the thick-based hairs.

(b) **Pterostylis turfosa.** (Bird Orchid.)

The specimens of this orchid each had a small rounded tuber situated an inch or so below the ground level from which arose a vertical underground stem. The underground stem gave rise to a new tuber at approximately the level of the old one and bore a number of small horizontal lateral roots at various points between the tubers and the ground level. No fungus was found in the roots or tubers examined, but the underground stem contained an intracellular fungus in considerable quantity. The stem below the ground level has large wart-like multicellular hairs, each with a number of elongated apical cells as in the Caladenias. Numerous vascular bundles occurred irregularly scattered through the central portion of the stem. The fungus occurred intracellularly in the cortical region. Fungal hyphae passed down the apical cells of the wart-like hairs into the soil.

(c) **Prasophyllum fimbria.**

Each specimen had a number of long, more or less, horizontal roots developed from the base of the flowering stem at the same region as the new tuber was produced. The new tuber was white, globular and comparatively smooth walled, while the old one was brown in colour, very hairy and fluted with a number of deep depressions. No fungus was found in the young or old tubers but a fungus occurred in the roots. Both young and old tubers contained abundant starch (staining red-brown with iodine) and possessed a well-defined stem structure. The roots showed a large-celled, thin-walled epidermis, an extensive cortex and only a small vascular region. The endotrophic mycorrhiza was only present a short distance into the cortex in most cases but occasionally extended in patches to the boundary of the vascular cylinder. The root hairs were simple outgrowths of the epidermal cells and provided a means of invasion for the fungal symbiont.

(d) **Diuris longifolia.**

Each specimen had a number of long thin roots developed from the base of the flowering stem. It also bore long, white, fleshy tuberous roots. These tuberous roots showed no fungal inhabitant but mycorrhiza occurred in the narrower roots in great abundance. The tuberous roots were similar in structure to the smaller roots but had a much larger cortex. The root hairs were normal on both types, and in the case of the invaded roots provided a readily availed-of means of entrance for the associated fungal inhabitant. No tubers were seen.

(e) **Lyperanthus nigricans.**

Each specimen of *Lyperanthus nigricans* had a stout vertical underground stem with numerous elongated narrow roots coming off laterally. The roots examined contained a somewhat scanty amount of a fungal symbiont. The endodermis was very noticeable in the roots and was composed of very large, angular, thick-walled cells. The root hairs were normal and provided passage ways for the mycorrhiza. Sections of the underground stem showed no fungus.

(f) **Thelymitra crinita.**

This orchid produces a number of more or less closely whorled, brownish, fleshy roots and a large white sub-cylindrical or pyriform tuber in a position closely adjacent to that occupied by the old one. Microscopic examination of roots revealed the presence of intracellular endotrophic mycorrhiza in considerable abundance and extending in some cases into the region of the faintly-marked endodermis. The root hairs were normal and many contained the mycorrhizal filaments. No fungus was present in either the new or the old tubers. A peculiar feature of the tubers was the absence of vasculars from the bulk of the tissue. What vasculars did occur were very small, few-celled, and located in a circular fashion *in a single continuous ring of several rows of small more or less oblong or ovoid cells.* This ring completely surrounded a well-marked "pith." Outside the ring the parenchymatous cells in the young tubers were densely packed with starch (staining red-brown with iodine), but the cells of the "pith," were devoid of starch.

(g) **Thelymitra longifolia.**

Except for minor differences the anatomy and mycorrhiza of the roots, and the anatomy of the tubers of T. *longifolia* was as described above for *T. crinita.*

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It is interesting to note that mycorrhiza was found in some portion of all the orchids examined, but never in the tubers or other vegetative propagating organs except in *Rhizanthella* (where a rhizome is the only vegetative structure produced). Moreover, it will be observed that in all cases the mycorrhiza was strictly intracellular, and in no single instance was the fungal inhabitant seen to enter or leave the plant infected except through the lumina of hairs produced either on the roots or on the stems. In *Rhizanthella*, *Caladenia* and *Pterostylis* special hair structures seem to have been evolved on the stems to ensure more adequate and certain invasion by the fungal inhabitants. *Rhizanthella* is an example of an orchid which is entirely dependant for its sustenance on the fungus with which its vegetative parts are intimately associated.

In conclusion it must be stated that this note does not pretend to have more than skimmed the surface of the subjects dealt with. A complete study of the morphology and mycorrhiza of any particular species mentioned above would take much more time than the writer has been able to give to the whole group listed. As regards the indigenous orchids of Western Australia the field is so far unexplored and much of intense interest awaits the future prospector. These lines will have served their purpose well if they simply bring to the notice of students of nature a field of research as yet untraversed and well worthy of close investigation.

My thanks are due to Mr. C. A. Gardner, Assistant Botanist, Department of Agriculture, for identification of the species examined, and to Mr. W. M. Carne, President of this Society for provision of the material, kindly assistance in every way, and helpful criticism.

EXPLANATION OF PLATES.

Plate X.—*Fig.* 1 : T.S. of underground portion of stem of *Caladenia flava* R. Br. X35. Note mycorrhiza in the amorphous condition in the cells of the cortex, but in the filamentous condition in the basal and apical cells of the wart-like hairs. Note also the two-rowed endodermis with spiral thickenings. A, wart-like hair ; B, apical cell containing fungal filaments ; C, apical cell without fungal filaments.

Fig. 2 : T.S. of rhizome of *Rhizanthella Gardneri*, Rogers. X6. Note vascular bundles arranged in a ring. Note also amorphous fungal masses in the cells around the peripheral region. A, wart-like hair with single long filamentous apical cell which provides a passage-way for the mycorrhiza in invading or leaving the host.

Plate XI.—*Fig.* 3 : T.S. of rhizome of *Rhizanthella Gardneri*. X25. A, wart-like hair with broken filamentous apical cell containing fungal hyphae ; B, region containing fungus in the filamentous condition ; C, region containing fungus in the amorphous condition ; D, vascular bundle.

Fig. 4 : T.S. of rhizome of *R. Gardneri* showing septate fungal filaments in the single apical cell of a thick-based hair. X 100. (This hair has a longer base and a shorter apical cell than is usual.) A, fungal filaments in host cells closely surrounding the host nuclei.

Plate XII.—*Fig.* 5 : T.S. of rhizome of *Rhizanthella Gardneri*. X 230. Note the much branched septate hyphae and the amorphous fungal masses. A, much enlarged deeply-staining nucleus of host cell closely appressed to an amorphous fungal mass. The nucleolus is deeply stained. Stain : Night Blue and Acid Fuschin following Flemming's weaker fixative ; B, Cell in which fungus is dominant. Note smallness and disorganisation of the host nucleus. C, Hypha passing through wall of host cell. Note peg-like process passing through the host wall.

Fig. 6 : Complete thick-based hair of *R. Gardneri*, X50. Note fungal hyphae within the lumen of the filamentous apical cell. Note also denseness of the entangled hyphae in the multi-cellular base and at the tip of the apical cell.

All drawings were made with the aid of a camera lucida.

PLATE X.

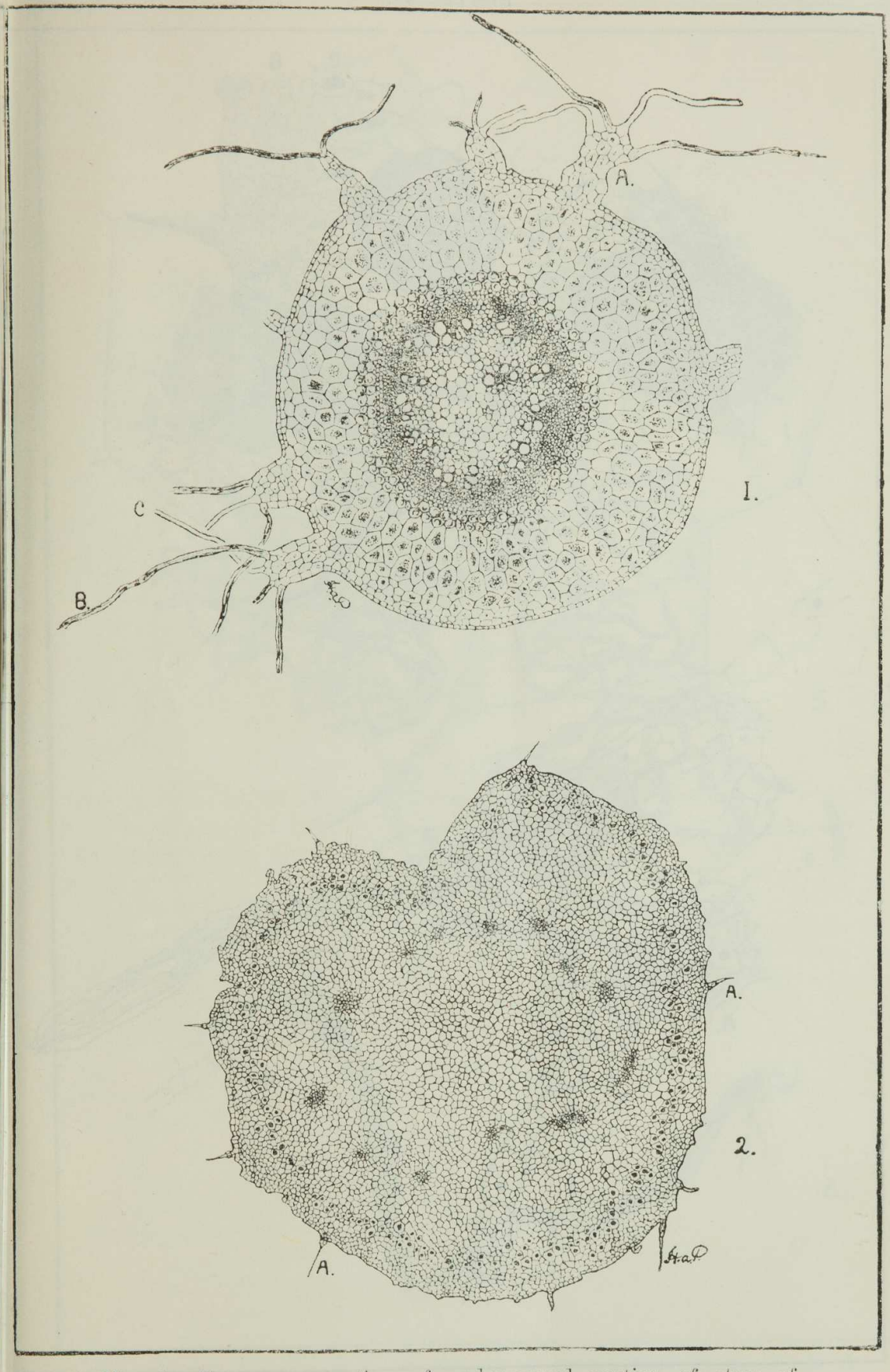

Fig. 1.—Transverse section of underground portion of stem of *Caladenia flava*. X 35.

Fig. 2.—Transverse section of rhizome of *Rhizanthella Gardneri*. X 6.

PLATE XI.

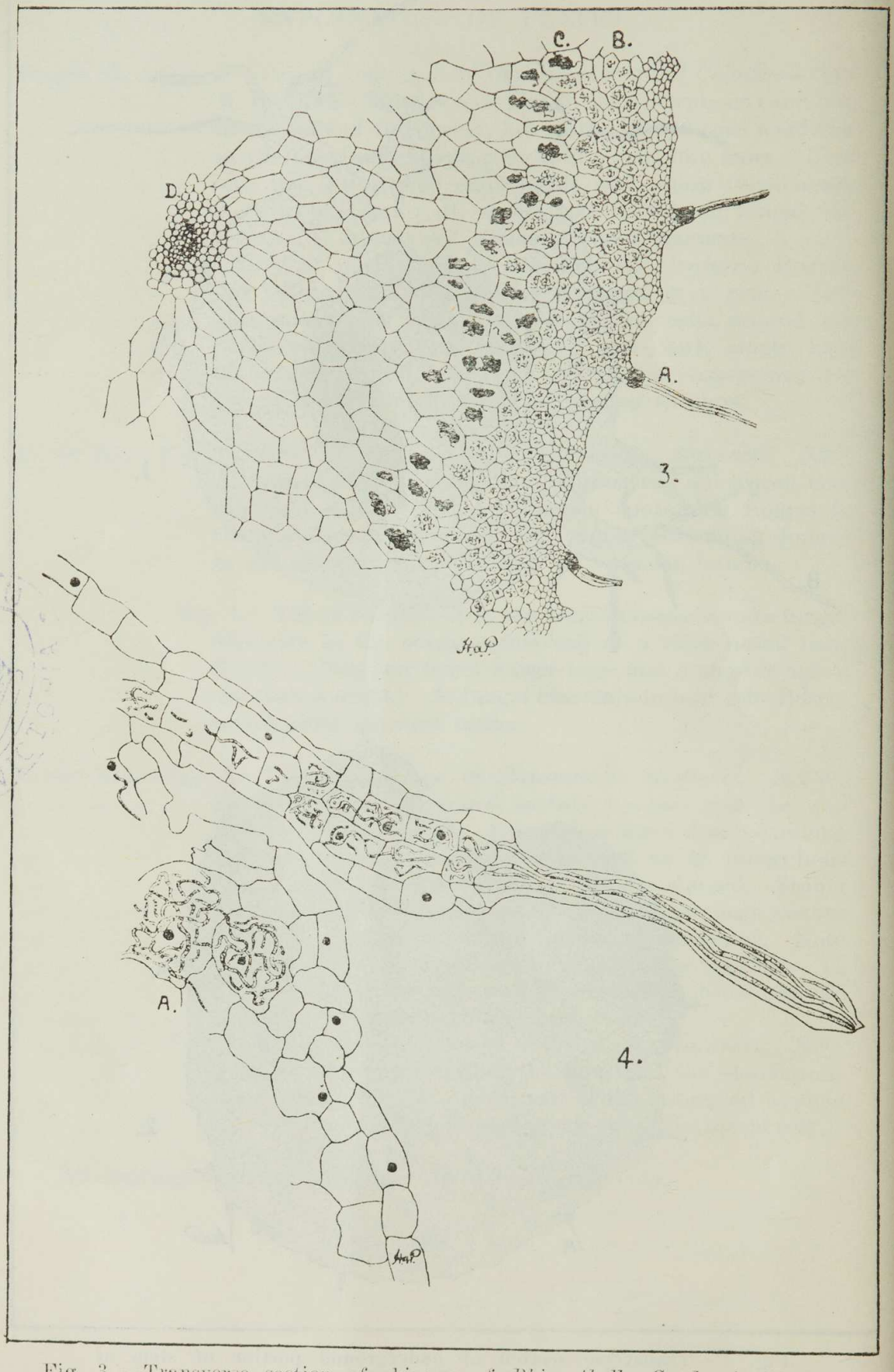

Fig. 3.—Transverse section of rhizome of *Rhizanthella Gardneri*. X 25.

Fig. 4.—T.S. of *R. Gardneri*, showing septate fungal filaments in apical cell of a thick-based hair. X 100.

PLATE XII.

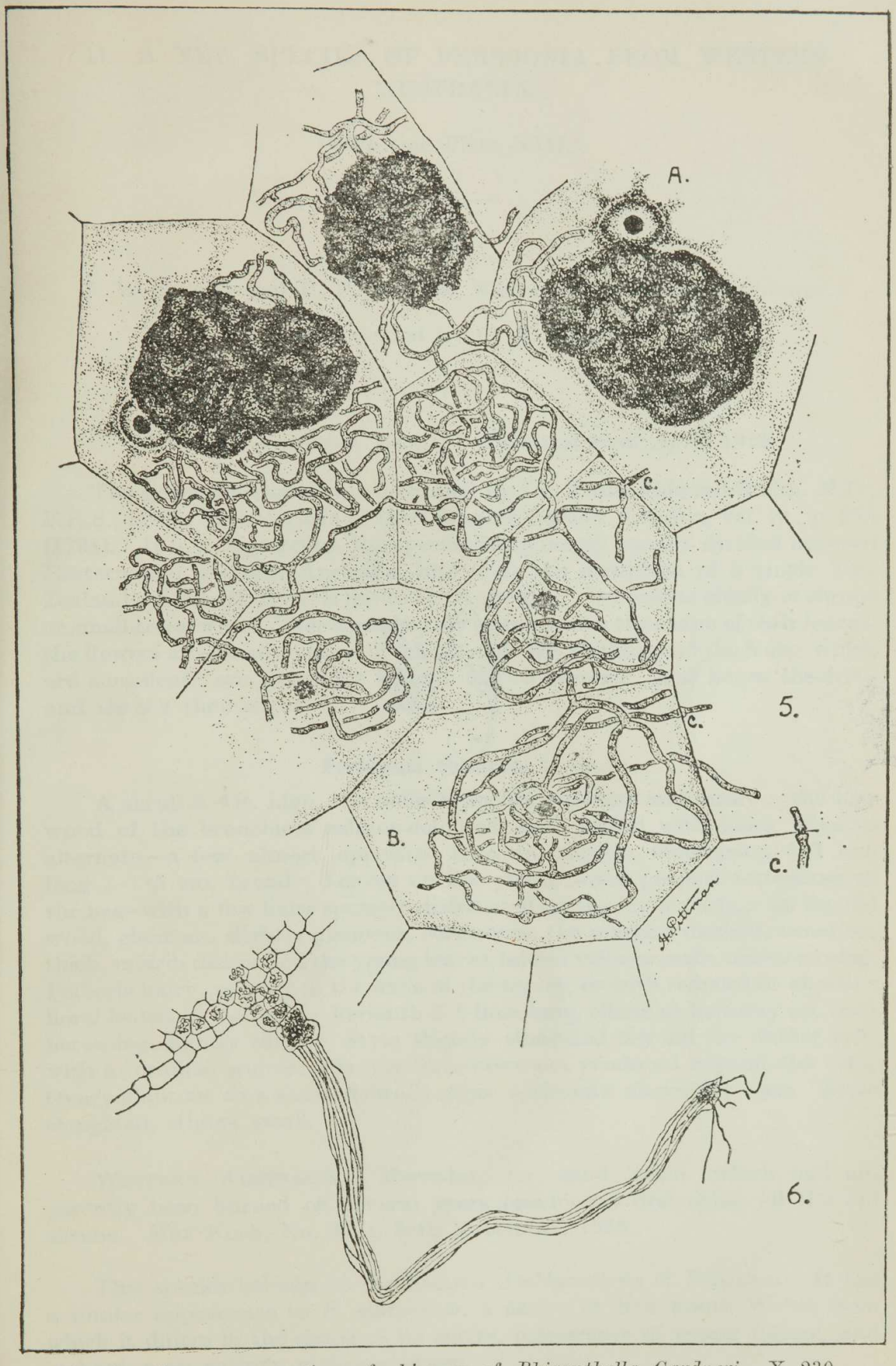

Fig. 5.—Transverse section of rhizome of *Rhizanthella Gardneri*. X 230.

Fig. 6.—Complete thick-based hair of *R. Gardneri*, showing numerous fungal filaments within it and also protruding into the soil. X 50.

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